PRODUCTION OF HOLLOW BACTERIA CELLULOSE MICROSPHERES FOR 3D CELL CULTURE SCAFFOLD

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The hollow microspheres are attractive components of artificial scaffold as extracellular matrix for cell proliferation.The main advantages include efficient fabrication, flexible self-assemble and more interface for cell attachment and more interspace for cell location provided by the hollow structure.[1] However, the available fabrication technique has limitations due to the complex chemical process and few material choices.[2] In this study, we have developed a microfluidic process to fabricate hollow nanofibrous microspheres using bacterial cellulose (BC), which is a promising biocompatible material with ultra-high purity and excellent mechanic strength.

Since BC fibers is not soluble in water and most organic solvents , it is difficult to manipulate directly in microfluidic system.Here, we encapsulated the cellulose producing bacteria strain *Gluconacetobacter xylinus* inside a double-layer template microparticle with alginate core and agarose shell generated by microfluidic control as shown in Fig. 1(a). The *G. xylinus* was encapsulated and confined in the agarose area during the long-time static culture due to the density differences between the agarose and alginate. The cellulose produced by *G. Xylinus* were then entangled to form the desired hollow structure. The agarose and alginate could be removed using high temperature and chemical process after the hollow BC microsphere formation as shown in Fig. 1(b).

Figure 2 shows the quantification of cellulose production process with different number of *G. xylinus* encapsulated inside the agarose template microparticles. Fluorescent brightener 28 was used to stain the cellulose fiber and the productivity was indicated by the fluorescent area ratio. The result shows that for a constant number of *G. xylinus* inside a single agarose microparticle, the cellulose production increased over time. After 12 days culture, compact cellulose microsphere could be collected. On the other hand, the amount and rate of cellulose production was depending on the initial number of *G. xylinus* encapsulated in a single agarose microparticle.

The hollow BC microspheres produced in the same batch were highly monodispersed with the same diameter and hollow structure as shown in Fig. 3(a-b). Fig. 5 (c) shows the SEM graphs of the cellulose fiber produced by *G. xylinus* cultured in agarose. Comparing with the bulk produced cellulose, the fiber generated was thinner and with the morphology of novel uniform. The hollow structure of the microsphere could also be controlled by encapsulating alginate particles with different sizes. Fig. 5(d-f) shows the hollow microspheres fabricated with the alginate cores of the diameter of 20 μm, 40 μm, 50 μm, respectively.

To examine the potential of application of the hollow BC microspheres for cell culture scaffold, the growth of PC-9 cell on three different types of artificial scaffold (BC membrane, packed BC microspheres and packed hollow BC microspheres) was carried out. We used confocal microscope to observe the growth of the cells in z direction. For the BC membrane, the cells are observed only attached to the surface of the scaffold. Meanwhile, cells are observed with a penetration depth of 65μm for the BC microsphere scaffold and deeper penetration depth of 95μm for hollow BC microspheres scaffold. The advantages on cell penetration depth of hollow BC micrspheres may induced by the larger gaps and flexibility of the scaffolds.

[1] D. R. Griffin, W. M. Weaver, P. O. Scumpia, D. Di Carlo and T. Segura, Nature Materials, 2015, 14, 737.
[2] X. H. Liu and P. X. Ma, *Nature materials*, 2011, **10**, 398.

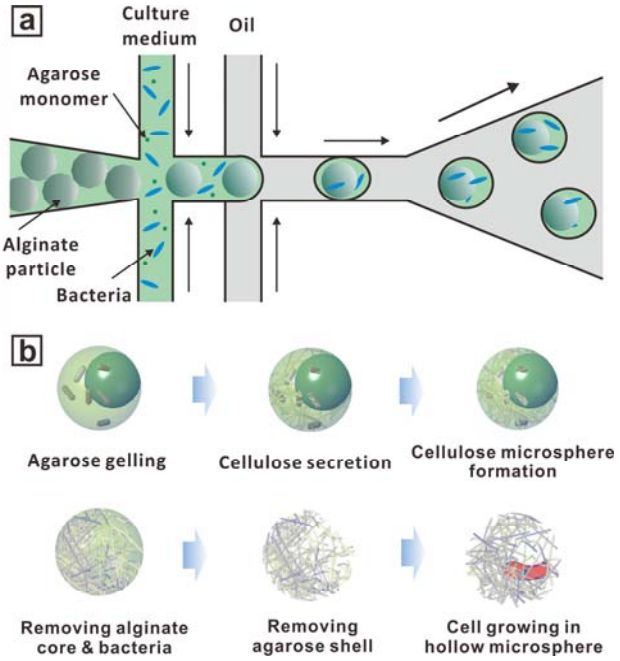

Fig.1 Schematics of the producing process of the hollow BC microsphere (a) generation of the double layer alginate core agarose shell microdroplet and (b) the agarose gelling, cellulose secretion, purification and application as cell growing scaffold.

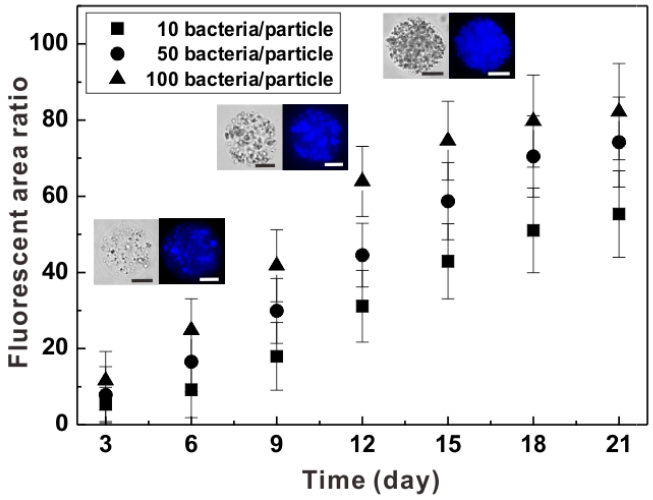

Fig. 2 Quantification of cellulose production by fluorescent area ratio indicated by fluorescent brightener 28. The inner graphs show the cellulose producing process in agarose particle on day 6, day 12 and day 18 under bright field or indicated by fluorescent brightener 28; scale bar: 25 μm.

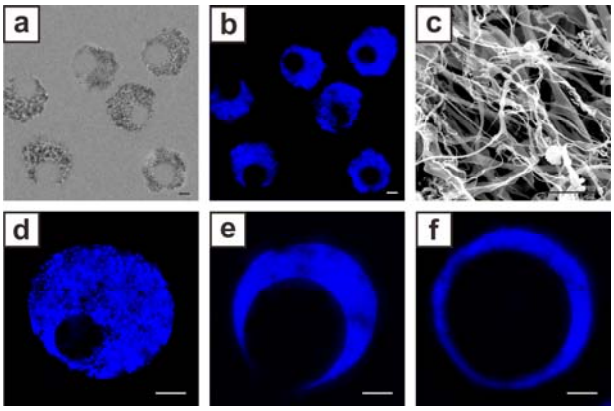

Fig. 3 Characterization of hollow BC microspheres (a-b) a batch of monodispersed hollow microspheres,(c) SEM graphs of the cellulose fiber produced by G. xylinus cultured in agarose and (d-f) hollow BC microspheres with different sizes of the alginate core of 20, 40 and 50 μm respectively; scale bar: 10 μm.

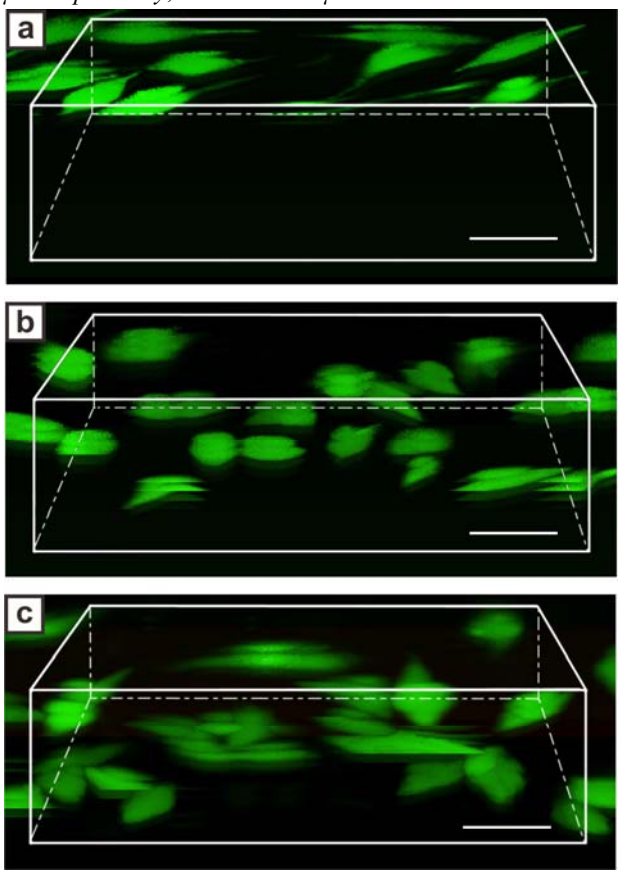

Fig. 4 3-D oblique view of fluorescence micrographs of PC-9 cell growth on three different types of BC scaffold showing the cells distribution and penetration depth.